This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

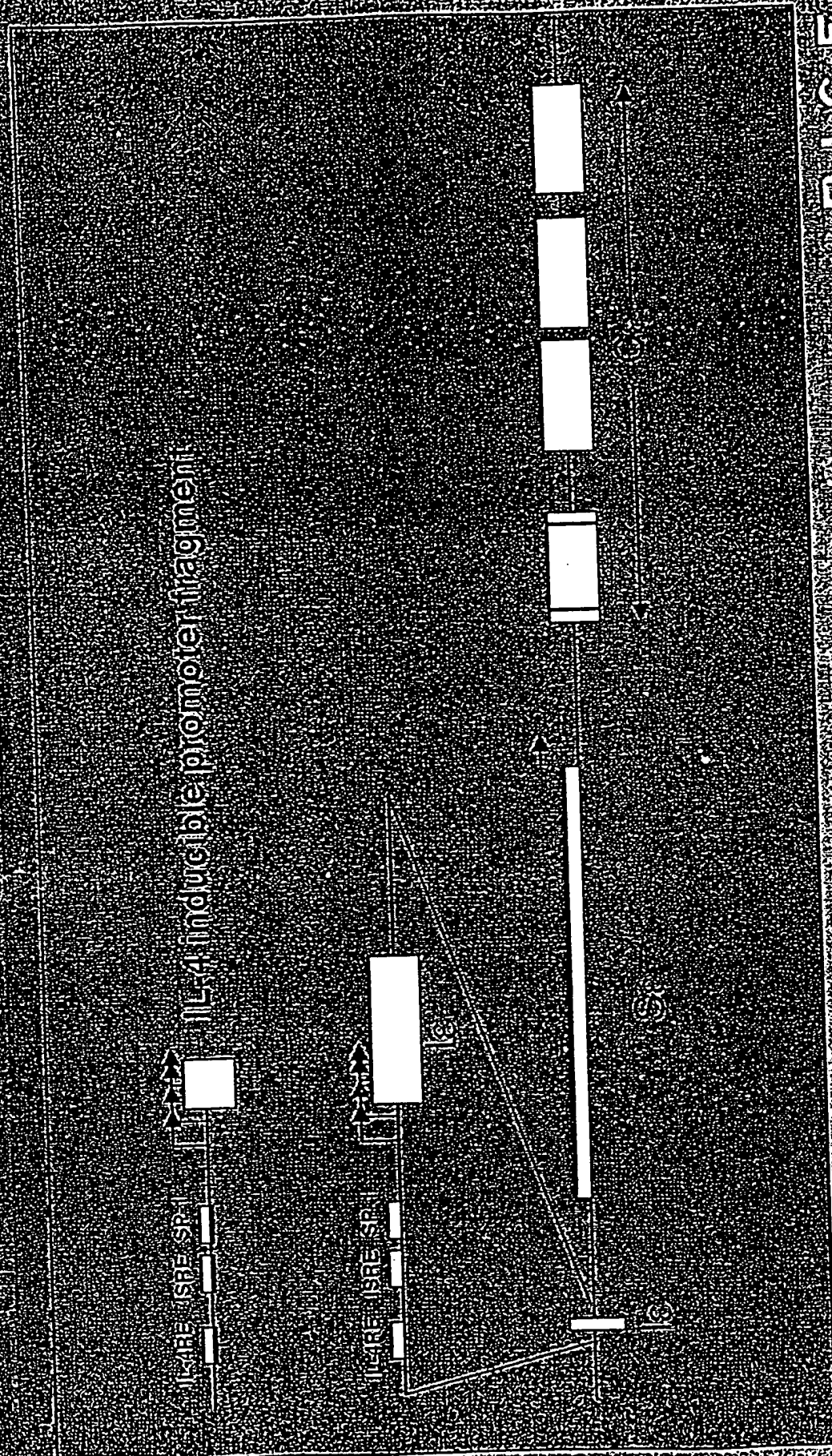Defects in the images may include (but are not limited to):

- BLACK BORDERS

- TEXT CUT OFF AT TOP, BOTTOM OR SIDES

- FADED TEXT

- ILLEGIBLE TEXT

- SKEWED/SLANTED IMAGES

- COLORED PHOTOS

- BLACK OR VERY BLACK AND WHITE DARK PHOTOS

- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

FIGURE 1A

CTCGAGGACAGTGACCTGGGAGTGAGTACAAGGTGAGGCCACCACTCAGGGT
GCCAGCTCCAAGCGGGTCACAGGGACGAGGGCTGCGGCCATCAGGAGGCCCT
GCACACACATCTGGGACACGCGCCCCGAGGGCCAGTTCACCTCAGTGCGCCT
CATTCTCCTGCACAAAAGCGCCCCATCCTTTCTTCACAAGGCTTTCGTGGAAG
CAGAGGCGTCGATGCCCAGTACCCTCTCCCTTTCCCAGGCAACGGGACCCCAA
GTTTGCTGACTGGGACCACCAAGCCACGCATGCGTCAAGAGTGAGAGTCCGG
GACCTAGGCAGGGGCCCTGGGGTTGGGCCTGAGAGAGAAGAGAACCTCCCCC
AGCACTCGGTGTGCATCGGTAGTGAAGGAGCCTCACCTGACCCCCGCTGTTGC
TCAATCGACTTCCCAAGAACAGAGAGAAAAGGGAACTTCCAGGGCGGCCCGG
GCCTCCTGGGGGTTCCCACCCCATTTTTAGCTGAAAGCACTGAGGCAGAGCTC
CCCCTACCCAGGCTCCACTGCCCGGCACAGAAATAACAACCACGGTTACTGAT
CATCTGGGAGCTGTCCAGGAATTC

FIGURE 1B

Low energy DNA folding of the Sε region

Highlighted areas: target sequences used in the one-hybrid screen
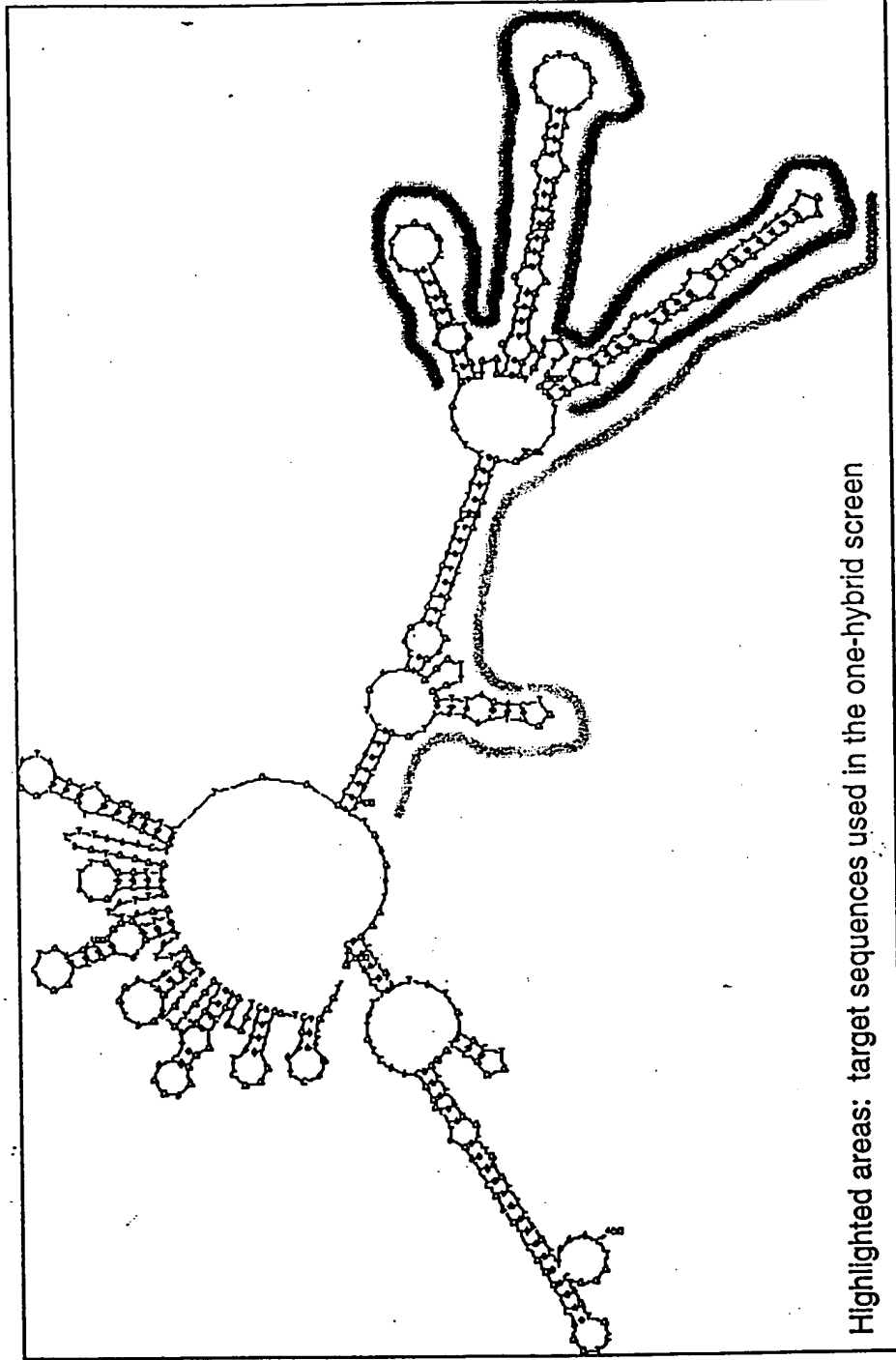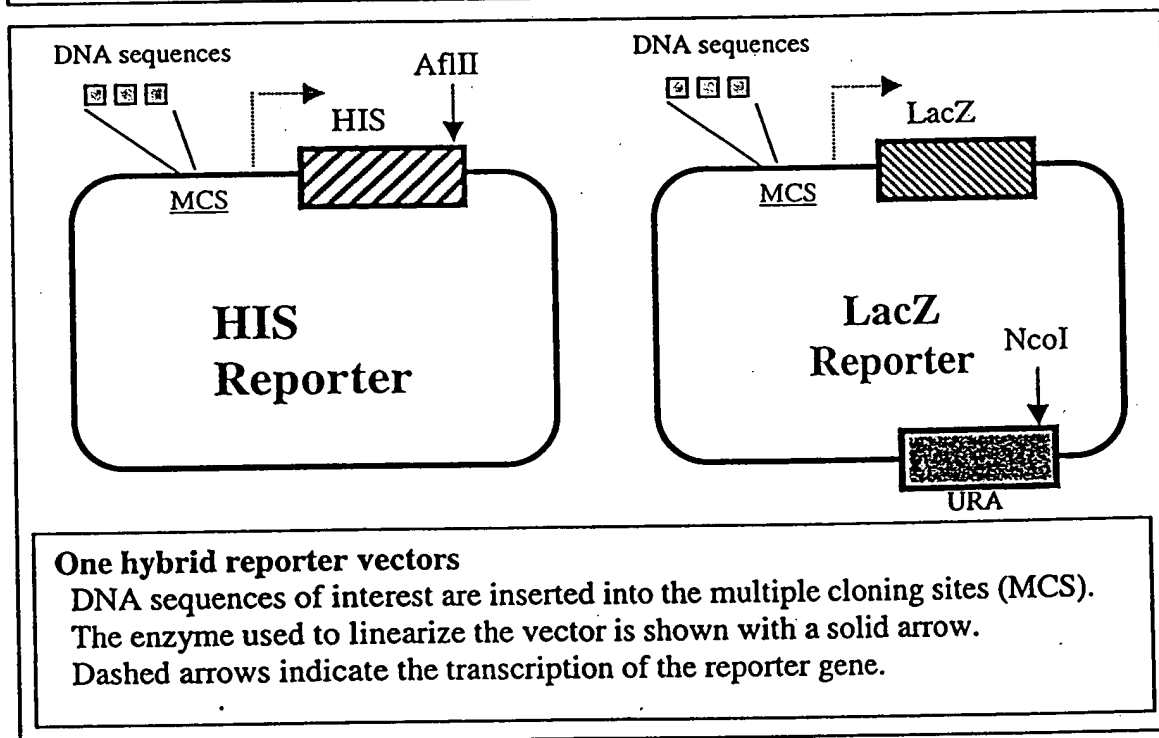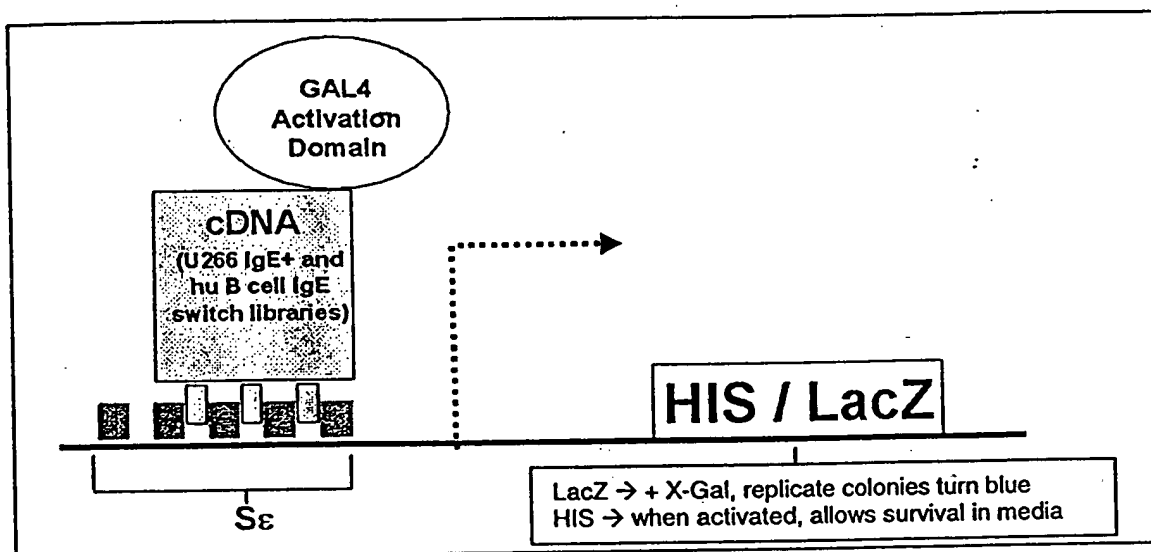
FIG 2A

Appendix E

FIGURE 2B

```
  1   GCTGGGCTAA ACTGGGCTAG CCTGAGCTGG GCTGAACTGG GCTGCTGGGC

 51   TGGACTGGGT AAGCTGGGCT GAGCTGGGTT GGGTGGAAAT GGGCTGAGCT

101   GAGCTAGGCT AAACTGGGTT TGGCTGGGCT GGGCTGGGCT GGG
```

FIGURE 2C

```
  1   GGTTTGGCTG GGCTGGGCTG GGCTGGGCTG GGTTCAGCTG AGCGGGTTGG

 51   GTTAGACTGG GTCAAACTGG TTCAGC
```

FIG 3

Appendix F   **Yeast One-Hybrid Screening**

GAL4
Activation
Domain

cDNA
(U266 IgE+ and
hu B cell IgE
switch libraries)

HIS / LacZ

Sε

LacZ → + X-Gal, replicate colonies turn blue
HIS → when activated, allows survival in media

DNA sequences

AflII

HIS

MCS

**HIS
Reporter**

DNA sequences

LacZ

MCS

**LacZ
Reporter**   NcoI

URA

One hybrid reporter vectors
DNA sequences of interest are inserted into the multiple cloning sites (MCS).
The enzyme used to linearize the vector is shown with a solid arrow.
Dashed arrows indicate the transcription of the reporter gene.

FIG 4

IL-4 Induction of Germline ε mRNA in the IgM+ B cell lines: CA-46, MC-116 and DND39

DND39 + IL-4

DND39 - IL-4

MC-116 + IL-4

MC-116 - IL-4

CA-46 + IL-4

CA-46 - IL-4

Neg cont.

246 bp

123 bp

Cells were incubated for 48 hrs in 300 U/ml of h-IL-4. RT-PCR was performed using primers specific for the germline ε exon and the 5'-end of the ε CH1 exon (predicted size ~ 200 bp).
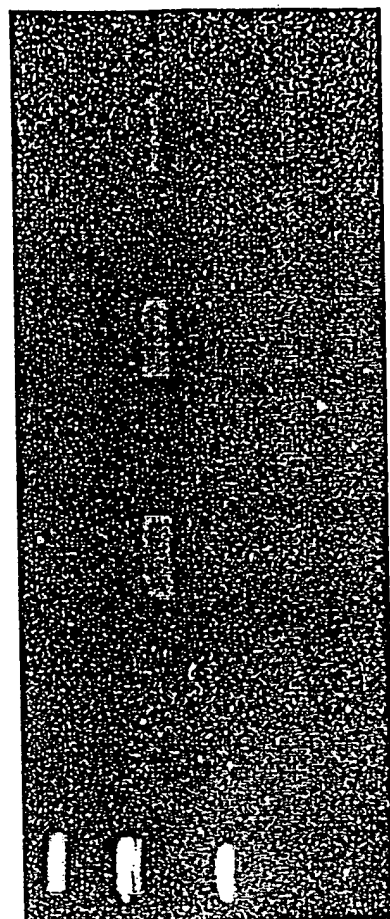
Appendix G

Approaches to generate germline ε promoter knock-in reporter cell lines

FIG 5

Knock-in constructs

A

B

C

D

CH1 — CH2 — CH3 — CH4

Cε

Sε

IL-4RE   IRE   SP-1

Iε

ε

GFP — CH2 — CH3 — CH4

BFP — CH2 — CH3 — CH4

Constitutive Promoter

BFP

SV40 promoter

GFP · Neo^r — CH2 — CH3 — CH4 — BFP

Lox P

IL-4 inducible IgM+ B cell lines are transfected. Under the influence of IL-4, GFP and/or BFP positive clones are isolated by FACS. Homologous recombination can be confirmed by PCR and/or Southern blot hybridization.

IL-4 inducible IgM+ B cell lines are transfected and selected with G418. Survivors are sorted for the lack of 3' BFP expression (deleted during homologous recombination). RT-PCR is performed to confirm homologous recombination. Those clones are transfected with *cre* to remove the neomycin resistance gene.

Il-4 RE, IL-4 responsive element
IRE, interferon responsive element
SP-1, SP-1 binding site
Iε, non-translated exon
Sε, switch region of ε
GFP, green fluorescent protein
BFP, blue fluorescent protein
CH1,2,3,4, constant region domain exons

Appendix A

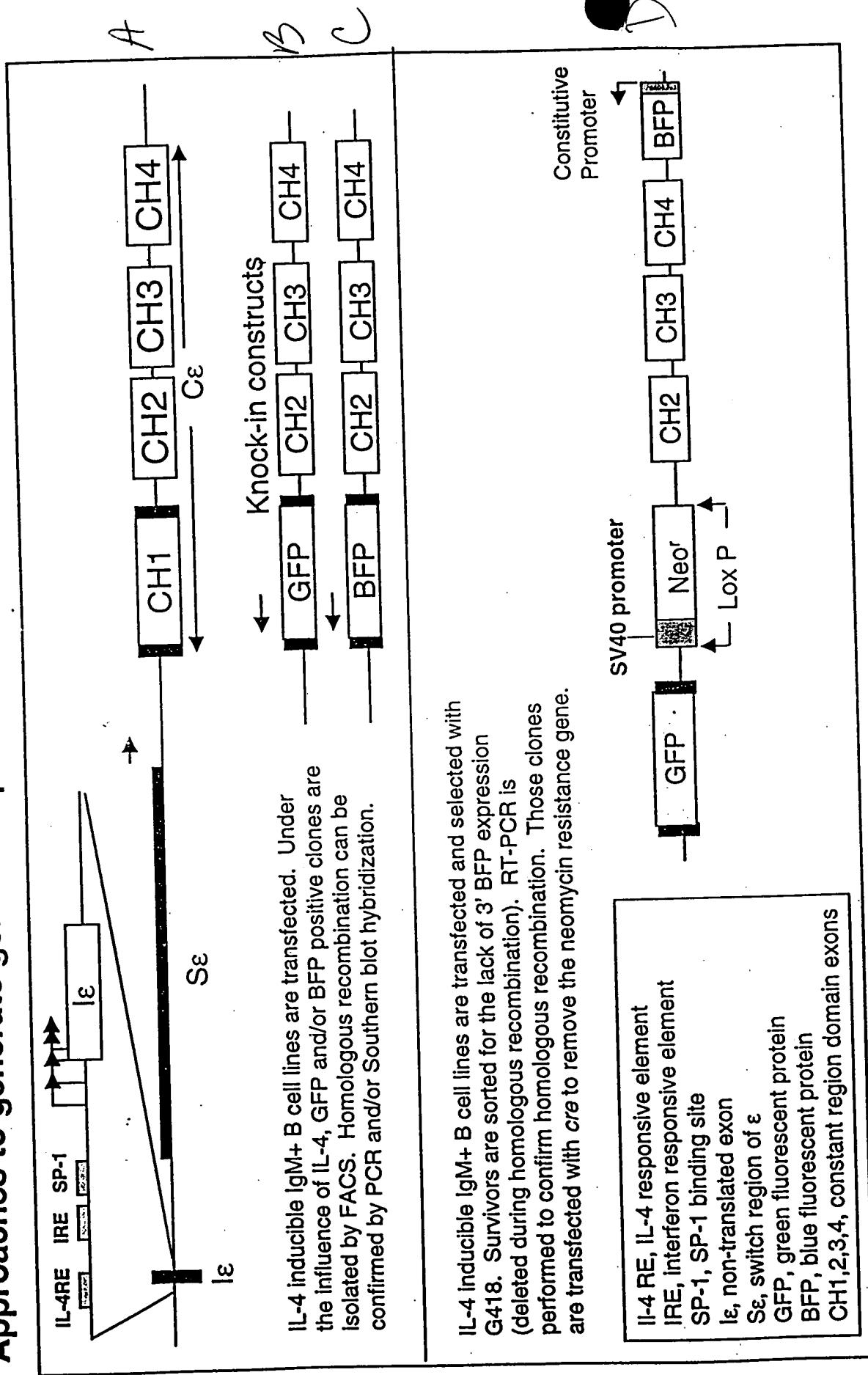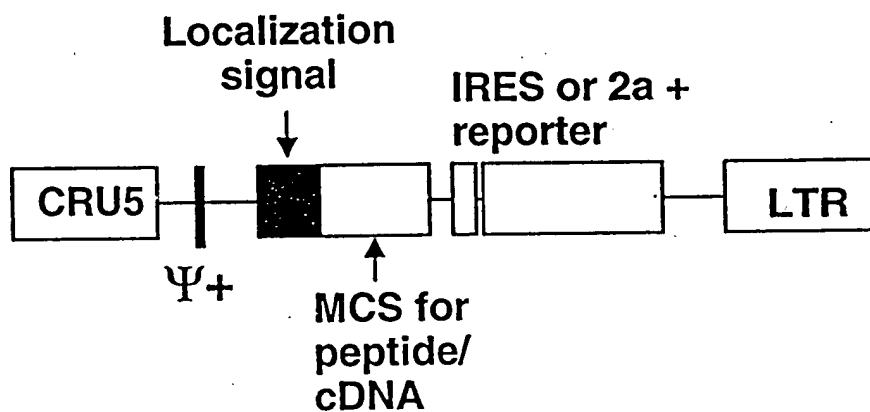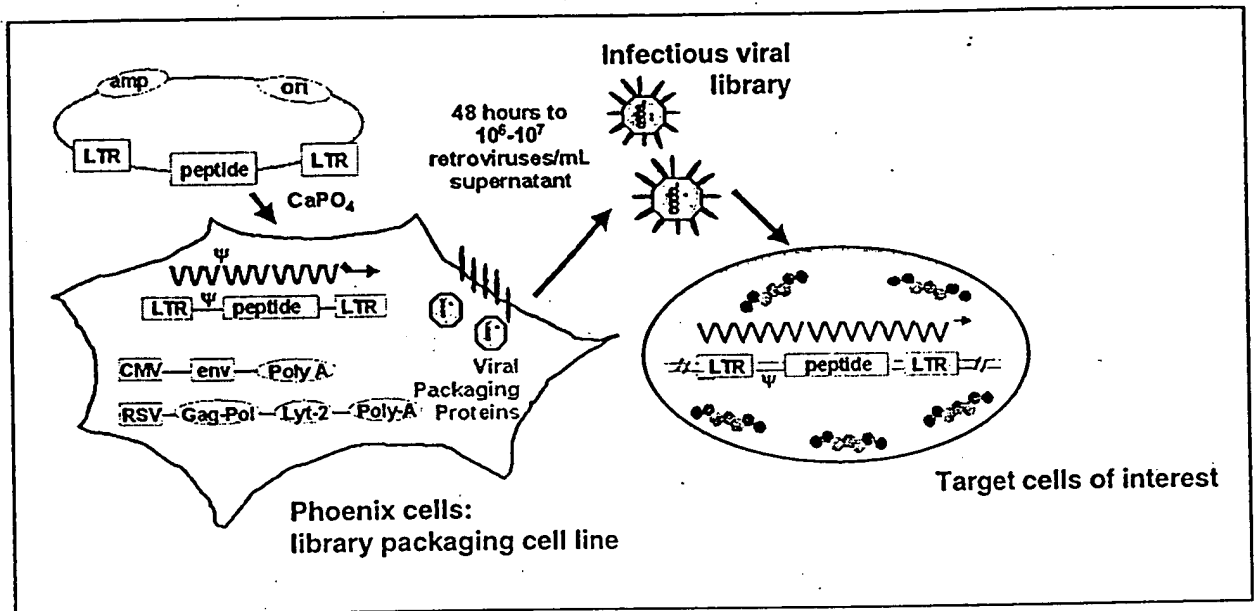**FIG 6**

Appendix I

Rigel Base Vector

All components are cassetted for flexibility

CRU5, modified LTR
LTR, long terminal repeat
ψ+, packaging signal
Localization signal: nuclear, cell membrane, granular
MCS, multiple cloning site
IRES, internal ribosome entry site
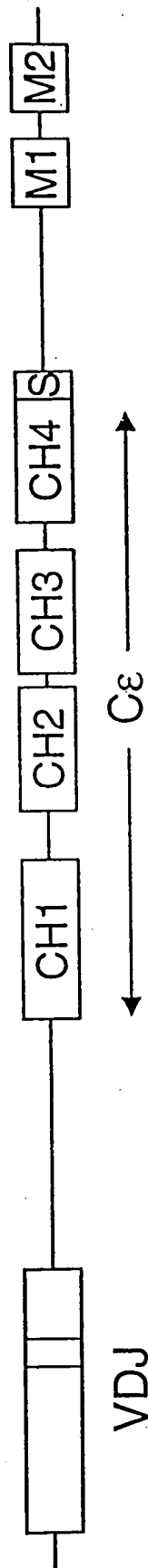2a, self-cleaving peptide

FIG -2

Appendix H

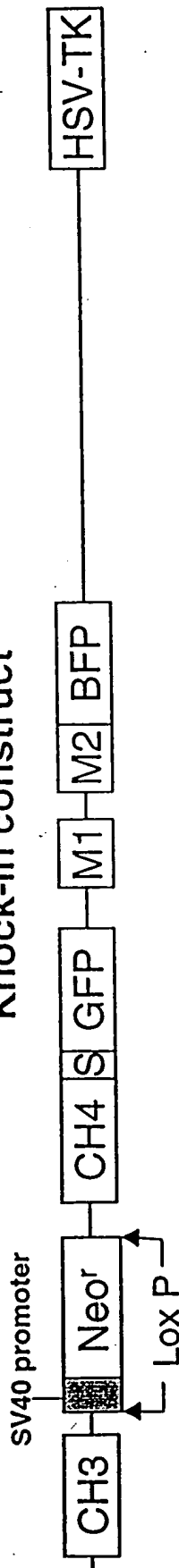## Protocol for Transfection of Phoenix Cells and Infection of Nonadherent Target Cells

Infectious viral library

48 hours to 10^6-10^7 retroviruses/mL supernatant

amp   ori

LTR   peptide   LTR

CaPO₄

ψ
WWWWWWW→
LTR ψ peptide LTR

CMV — env — Poly A

RSV — Gag-Pol — Lyt-2 — Poly-A

Viral Packaging Proteins

Phoenix cells:
library packaging cell line

WWWWWWWWWW→
LTR ψ peptide LTR

Target cells of interest

FIG 8

ε heavy chain GFP/BFP knock-in cell line

U266 ε heavy chain

Knock-in construct

S, secretory exon
GFP, green fluorescent protein
BFP, blue fluorescent protein
Neo^r, neomycin resistance gene
VDJ, V region exon
CH1,2,3,4, constant region domain exons
M1, M2, membrane exons
HSV-TK, Herpes Simplex virus-Thymidine Kinase

U266 cells are transfected and selected with G418. Survivors are treated with ganciclovir (HSV-TK deleted during homologous recombination). RT-PCR is performed to confirm homologous recombination. Those clones are transfected with *cre* to remove the SV40 neomycin resistance gene.
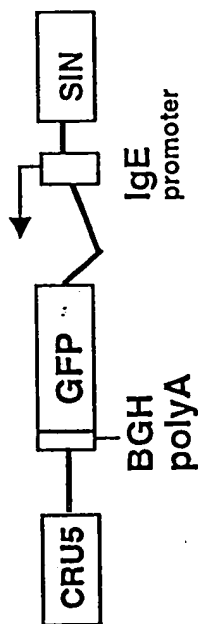
Appendix D

FIG 9

IL-4 Inducible ε Promoter Reporter Cell Line

Reporter construct

IL-4 induced reporter

Control infected — 1.21%

0 units/mL IL-4 — 2.84%

200 units/mL IL-4 — 92.36%

Number

GFP expression →
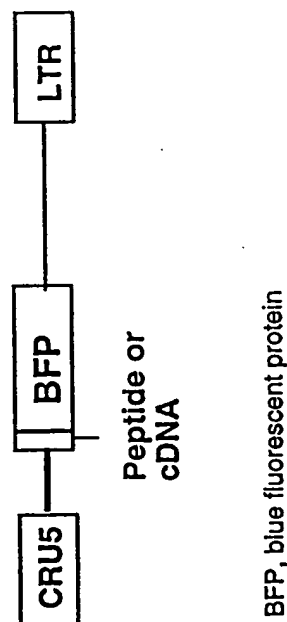
CRU5 — GFP — BGH polyA — IgE promoter — SIN

CRU5, hCMV promoter plus R and U5 regions of LTR
GFP, green fluorescent protein
BGH poly A, bovine growth hormone poly-adenylation signal
SIN, self-inactivating LTR

Rigel, Inc.   Confidential
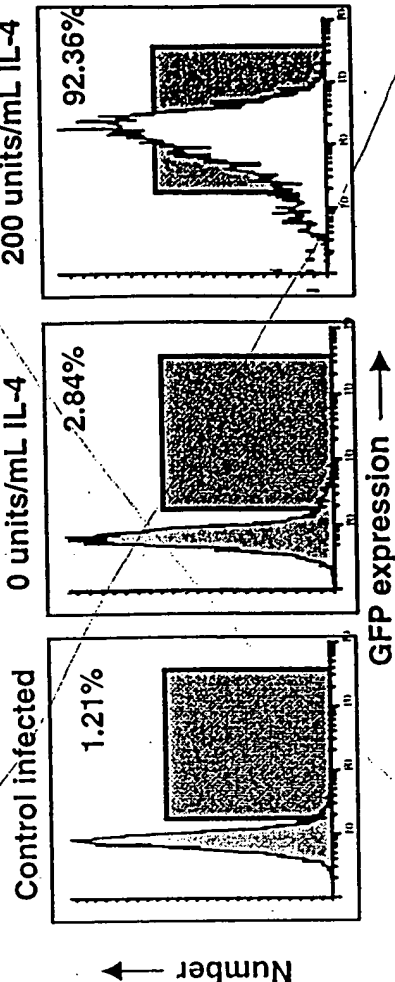
Reporter Line Infected with BFP Construct

FACS profile of cells with both reporter and peptide library

NQ IL-4 — 4.26'   0.8   57/34   0.6

100 U/ml IL-4 — 0.9   42.64   4.6'5'

BFP expression

GFP expression →

Library construct

CRU5 — BFP — LTR

Peptide or cDNA

BFP, blue fluorescent protein

Appendix C

18

FIG 10
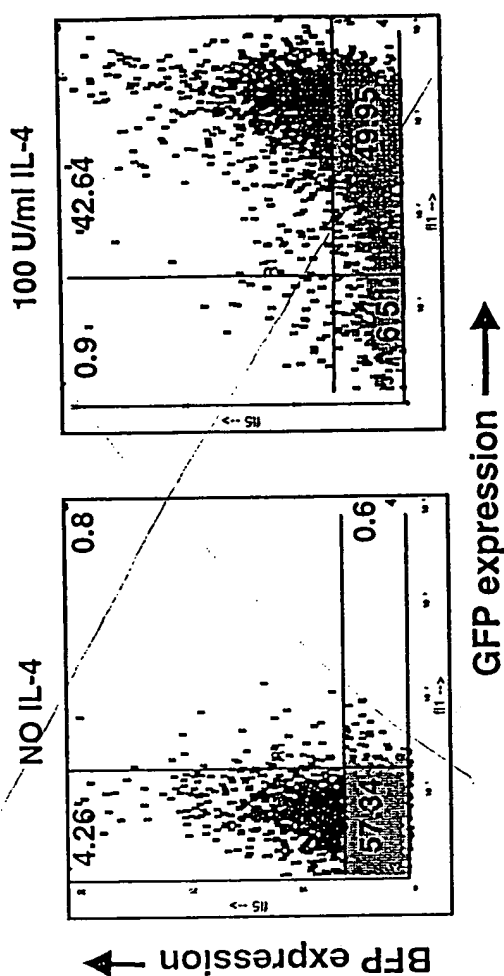
Screen for Peptide Inhibitors of the Germline ε Promoter

Infected cells

+ selection agent →

+ IL-4
+ puromycin →

Removal of leaky cells

Survival of IL-4 inducible clones

+ library
+ IL-4
+ selection agent →

Survival of peptide inhibited clones

Survival Construct

SIN | LTR — Ψ+ → | IL-4 inducible ε germline promoter → | Position 1 | 2a → | Puromycin^r or GFP → | SIN | LTR

Position 1
- FAS chimeric receptor *
  - * (mouse FASr external / mouse CD8 external + human transmembrane and cytoplasmic domains)
- HSV-TK
- P450 2B1
- p21 peptide

Death Inducer + Selection Agent
- αFAS
- ganciclovir
- cyclophosphamide
- none (self selection)

SIN, self-inactivating LTR
LTR, long terminal repeat

All components are cassetted for flexibility

Appendix D

1-845 CMV promoter/R/U5 5' LTR
1322   GAG ATG-ATC mutation
850-2100 extended ψ region
2146-2173 two Bstx1 peptide cloning sites
2205-2723 ECMV IRES (cloned as EcoR1/Mscl fragment from
     pCITE-4a [Novagen])
2746-3465 GFP coding region
3522-4115 3' LTR
4122-6210 pGEM backbone (pUC origin, ampR)

```
ATCACGAGGCCCTTTCGTCTTCAAGAACAGCTTTGCTCTTAGGAGTTTCCTAATACATCC
CAAACTCAAATATATAAAGCATTTGACTTGTTCTATGCCCTAGTTATTAATAGTAATCAA
TTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA
ATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATG
TTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT
AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG
TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC
CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGC
AGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCA
TTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA
ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAA
GCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACT
GAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGT
CTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTT
CATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCG
GGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGA
TTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG
TGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCGG
GGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGACTCTTTGGTG
CACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCC
CGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCGCCGCGCGTCTTGTCT
GCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATA
TCGGCCCGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATG
TCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCT
GCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAACCGAG
ACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACACCCAGACC
AGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGC
CCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTG
AACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAG
GCGCCCCCATATGGCCATATGAGATCTTATATGGGGCACCCCCGCCCCTTGTAAACTTCC
CTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCTCACTTACAGGCTC
TCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACTGG
ACCGACCGGTGGTACCTCACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACC
AGACTAAGAACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCA
CCCGCCCTCAAAGTAGACGGCATCGCGCTTGGATACACGCCGCCCACGTGAAGGCTGCCGA
CCCCGGGGGGTGGACCATCCTCTAGACTGCCGGATCTCGAGGGATCCACCACCATGGACCC
CCATTAAATTGGAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGAATTAATTCC
```

GGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTG
TCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGT
TGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAG
CGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGC
CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGA
TAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATG
CCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACAT
GTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCT
TTGAAAAACACGATGATAATATGGGGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCG
AGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGA
AGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGA
CCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCA
AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA
ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGC
TGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT
ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT
TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA
ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGT
CCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGA
CCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCTCGACGA
TAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTA
GGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGA
GAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACA
GGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTG
AATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAA
CAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTC
CAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCG
CTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCC
TCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAA
ACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGA
GTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTCCGACTTGTGGTCTCGCTGCCTTGG
GAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTCACATGCAGCATGTAT
CAAAATTAATTTGGTTTTTTTTCTTAAGTATTTACATTAAATGGCCATAGTTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGCTCTTCCGCTTCCTCGCT
CACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGC
GGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGG
CCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCG
CCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGG
ACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGAC
CCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCA
TAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGT
GCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTC
CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAG
AGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACAC
TAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGT
TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAA
GCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGG
GTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAA
AAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTGCGCAAATCAATCTAAAG
TATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTC
AGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTAC
GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTC
ACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGG

TCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG
TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTC
ACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTAC
ATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAG
AAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTAC
TGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG
AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGC
GCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACT
CTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTG
ATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAA
TGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTT
TCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATG
TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTC

FIGURE 11B-1

1-845 CMVpormoter/R/U5 5' LTR
1322 GAG ATG-ATC mutation
850-2100 extended □ region
2151-2865 GFP coding region
2866-2894 GGGSGGG linker
2895-2952 FMDV 2a cleavage sequence
2953-3004 Bstx1/Bstx1/HinD3/Hpal/Sall/Not1 polylinker
3052-3645 3' LTR
3652-5715 pGEM backbone (pUC origin, ampR)

ATCACGAGGCCCTTTCGTCTTCAAGAACAGCTTTGCTCTTAGGAGTTTCCTAATACATC
CCAAACTCAAATATATAAAGCATTTGACTTGTTCTATGCCCTAGTTATTAATAGTAATC
AATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGG
TAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG
TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTT
ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTA
TTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGG
GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCG
GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTC
TCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA
AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGGA
GGTCTATATAAGCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTC
CTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCA
TCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGT
CAGCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACC
ACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTC
TAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGT
ATCTGGCGGACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAG

ACGTCCCAGGGACTTCGGGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGAT
CGTTTTGGACTCTTTGGTGCACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGA
CGAGAACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAA
GCCGCGCCGCGCGTCTTGTCTGCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTG
TTTCTGTATTTGTCTGAAAATATCGGCCCGGGCCAGACTGTTACCACTCCCTTAAGTTT
GACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCA
AGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGG
CCGCGAGACGGCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTC
ACCTGGCCCGCATGGACACCCAGACCAGGTCCCTACATCGTGACCTGGGAAGCCTTGG
CTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCTT
CCTCCATCCGCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTC
CCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTAT 3ʰ - 1770
ATGGGGCACCCCGCCCCTTGTAAACTTCCCTGACCCTGACATGACAAGAGTTACTAAC
AGCCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAG
ACCTCTGGCGGCAGCCTACCAAGAACAACTGGACCGACCGGTGGTACCTCACCCTTACC 2
GAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGG
⁵ AAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCTCAⒶⒶGTAGACGGCATCGC 2006
AGCTTGGATACACGCCGCCCACGTGAAGGCTGCCGACCCCGGGGGTGGACCATCCTCTA
GACTGCCGGATCTCGAGGGATCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG
GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTC
CGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCA
CCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAG 40 - 2360
TGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCC
CGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC
GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATC
GACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCA
CAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC
GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC
ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCG
CCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGAATTCGGAGGTGGCAGCGGTGGC
GGTCAGCTGTTGAATTTTGACCTTCTTAAACTTGCGGGAGACGTCGAGTCCAACCCTGG 5ʰ - 2650
GCCCACCACCACCATGGAAGCTTCCATTAAATTGGTTAACGTCGACGCGGCCGCTCGAC
GATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCT
GTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAA
CTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCC
AAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAA
CAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGG
CCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCA
GATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCA
ATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGC
CCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGT
GTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAG
GGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTCCGACTTGTGGT
CTCGCTGCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTCA 2772
CATGCAGCATGTATCAAAATTAATTTGGTTTTTTTTCTTAAGTATTTACATTAAATGGC 69 - 3776
CATAGTTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGCT

CTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTA
TCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAA
GAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGG
CGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAG
AGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCT
CGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTT
CGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC
GTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTT
ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAG
CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTG
AAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT
GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG
CTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCT
CAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACG
TTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT
AAAAATGAAGTTTGCGCAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGT
TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCC
CCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATA
AACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCAT
CCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC
GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCT
TCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAA
AAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGT
TATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGA
TGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCG
ACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTT
TAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCG
CTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTT
TACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGG
GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGA
AGCATTTATCAGGGTTATTGTCTCATGACATTAACCTATAAAAATAGGCGT

FIGURE 11C-1

1-845 CMVpormoter/R/U5 5' LTR
1322 GAG ATG-ATC mutation
850-2100 extended Ξ region
2146-2173 two Bstx1 peptide cloning sites
2173-2214 EoR1/Apa1/Hpa1/Not1 polylinker
2262-2855 3' LTR
2855-4901 pGEM backbone (pUC origin, ampR)

ATCACGAGGCCCTTTCGTCTTCAAGAACAGCTTTGCTCTTAGGAGTTTCCTAATACATCCCAAACTCAAAT-
ATATAAAGCATTTGACTTGTTCTATGCCCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAG
CCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCG
CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG
TGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT
GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTG
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTG
GATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCAC
CAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGT
ACGGTGGGAGGTCTATATAAGCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTC
CGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCT
CGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTC
GTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTTA- 1) 923
TCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACT
AGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGT
CCCAGGGACTTCGGGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGACTCTTTG
GTGCACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCCCGCCTCCG
TCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCGCCGCGCGTCTTGTCTGCTGCAGCATCGTTCTGTG
TTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATATCGGCCCGGGCCAGACTGTTACCACTCCCT
TAAGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAG- 20 -1420
AGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTT
TAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACACCCAGACCAGG
TCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACACCCT
AAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCG
ATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGG -15 -1775
CACCCCCGCCCCTTGTAAACTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCT
CACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACT 28 -1448
GGACCGACCGGTGGTACCTCACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGA- 2
ACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCTCAAAGTAGACGGC
ATCGCAGCTTGGATACACGCCGCCCACGTGAAGGCTGCCGACCCCGGGGGTGGACCATCCTCTAGACTGCC- 3' -2130
GGATCTCGAGGGATCCACCACCATGGACCCCCATTAAAATTGGAATTCGGGGCCCAAGCTTTGTTAACGTCG
ACGCGGCCGCCGTCGACGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCC
CACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAA
TAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTA
AGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT
GGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGT
TTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTA
ACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACC
CCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTG

CAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGC
GGGGGTCTTTCATTTCCGACTTGTGGTCTCGCTGCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGT
CAGCGGGGGTCTTCACATGCAGCATGTATCAAAATTAATTTGGTTTTTTTTCTTAAGTATTTACATTAAAT
GGCCATAGTTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGCTCTTCCGCTT
CCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTA
ATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAG
GAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATC
GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC
CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGT
GGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG
TGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTA
AGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGC
TACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGC
TGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGT
GGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC
TACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGA
TCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTGCGCAAATCAATCTAAAGTATATATGAGTAAACT
TGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA
TGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAG
CGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG
TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGT
TTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAA
AAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGT
TATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACT
CAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAAT
ACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAG
GATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTA
CTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACA
CGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCAT
GACATTAACCTATAAAAATAGGCGT

(1) C12ScFas Survival Construct

C12ScFas: epsilon-cFas(CD95)-Ires-Hygro-BGH PolyA put into C12s vector backwards so
that no leaky transcription happens through the cmv promoter.

```
atcacggaggccctttcgtcttcaagaacagctttgctcttaggagtttcctaatacatcccaaactcaaatatataaagc  -40
atttgacttgttctatgccctagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccg
cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgaccccccgcccattgacgtcaataatgacgtatg
ttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagta
catcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagta
catgaccttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggc
agtacatcaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttg
ttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcatgt
acggtgggaggtctatataagcagagctcaataaaagagcccacaacccctcactcggggcgccagtcctccgattgact
gagtcgcccgggtacccgtgtatccaataaaccctcttgcagttgcatccgacttgtggtctcgctgttccttgggaggg
tctcctctgagtgattgactacccgtcagcggggggtctttcatttggggggctcgtccgggatcggggagacccctgcccag
ggaccaccgacccaccaccgggaggtaagctggccagcaacttatctgtgtctgtccgattgtctagtgtctatgactga
ttttatgcgcctgcgtcggtactagttagctaactagctctgtatctgggaccgtggtggaactgacgagttcggaa
cacccggccgcaaccctgggagacgtcccagggacttcggggggccgtttttgtggcccgacctgagtccaaaaatcccga  15-1200
tcgtttggactctttggtgcacccccccttagaggagggatatgtggttctggtaggagacgagaacctaaaacagttcc
cgcctccgtctgaattttttgctttcggtttgggaccgaagccgcgccgcgcgtcttgtctgctgcagcatcgttctgtgt
tgtctctgtctgactgtgtttctgtatttgtctgaaaatatgggcccgggccagactgttaccactcccttaagtttgac
cttaggtcactggaaagatgtcgagcggatcgctcacaaccagtcggtagatgtcaagaagagacgttgggttaccttct
gctctgcagaatggccaaccctttaacgtcggatggccgcgagacggcacctttaaccgagaccctcatcacccaggttaag
atcaaggtcttttcacctggcccgcatggacacccagaccaggtcccctacatcgtgacctgggaagccttggctttttga
ccccctccctgggtcaagccctttgtacacccctaagcctccgcctcctcttcctccatccgcccgtctctccccttg
aacctcctcgttcgacccgcctcgatcctcccttatccagccctcactccttctctaggcgcccccatatggccatat
gagatcttatatggggcaccccgcccccttgtaaacttccctgaccctgacatgacaagagttactaacagccctctct
ccaagctcacttacaggctctctacttagtccagcacgaagtctggagacctctggcggcagcctaccaagaacaactgg
accgaccggtggtacctcacccttaccgagtcggcgacacagtgtgggtccgccgacaccagactaagaacctagaacct  16-2000
cgctggaaaaggaccttacacagtcctgctgaccacccccaccgccctcaaagtagacgggcatcgcagcttggatacacgc
cgcccacgtgaaggctgccgaccccgggggtggaccatcctctagactgccGGATCTCGAGGGATCCTCCCCAGCATGCC
```

TGCTATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAA

TGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGG

GCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGtCTAGCTTGCCAAACCTACAGGTGGGGTCTTTCATTCCC

```
CCCTTTTTCTGGAGACTAAATAAAATCTTTTATTTTatcgatagatcccggtcggcatctactctattccttttgccctcg
gacgagtgctggggcgtcggtttccactatcggcgagtacttctacacagccatcggtccagacggccgcgcttctgcgg
gcgatttgtgtacgcccgacagtcccggctccggatcggacgattgcgtcgcatcgaccctgcgcccaagctgcatcatc
gaaattgccgtcaaccaagctctgatagagttggtcaagaccaatgcggagcatatacgcccggagccgcggcgatcctg  15-2000
caagctccggatgcctccgctcgaagtagcgcgtctgctgctccatacaagccaaccacggcctccagaagagatgttg
gcgacctcgtattgggaatccccgaacatcgcctcgctccagtcaatgaccgctgttatgcggccattgtccgtcaggac
attgttggagccgaaatccgcgtgcacgaggtgccggacttcggggcagtcctcggcccaaagcatcagctcatcgagag
cctgcgcgacggacgcactgacggtgtcgtccatcacagtttgccagtgatacacatggggatcagcaatcgcgcatatg
aaatcacgccatgtagtgtattgaccgattccttgcggtccgaatgggccgaacccgctcgtctggctaagatcggccgc
agcgatcgcatccatggcctccgcgaccggctgcagaacagcgggcagttcggtttcaggcaggtcttgcaacgtgacac
cctgtgcacggcgggagatgcaataggtcaggctctcgctaaattccccaatgtcaagcacttccggaatcgggagcgcg
gccgatgcaaagtgccgataaacataacgatctttgtagaaaccatcggcgcagctatttacccgcaggacatatccacg
ccctcctacatcgaagctgaaagcacgagattcttcgccctccgagagctgcatcaggtcggagacgctgtcgaactttt
cgatcagaaacttctcgacagacgtcgcggtgagttcaggctttttcatggtattatcatcgtgtttttcaaaggaaaac  46
cacgtccccgtggttcgggggggcctagacgtttttttaacctcgactaaacacatgtaaagcatgtgcaccgaggcccag
atcagatcccatacaatggggtaccttctgggcatccttcagccccttgttgaatacgcttgaggagagccatttgactc
tttccacaactatccaactcacaacgtggcactggggttgtgccgcctttgcaggtgtatcttatacacgtggcttttgg  44-3840
ccgcagaggcacctgtcgccaggtggggggttccgctgcctgcaaagggtcgctacagacgttgtttgtcttcaagaagc
ttCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGAGGGGAAAGACCCctagactaga
```

```
ccaagctttggatttcatttctgaagtttgaattttctgagtcactagtaatgtccttgaggatgatagtctgaatttttc
tctgcaagagtacaaagattggctttttttgagatctttaatcaatgtgtcatacgcttctttctttccatgaagttgatg
ccaattacgaagcagttgaactttctgttctgctgtgtcttggacattgtcattcttgatctcatctattttggcttcat
tgacaccattctttcgaacaaagcctttaacttgacttagtgtcatgactccagcaatagtggtgatatatttactcaag
tcaacatcagataaatttattgccactgtttcaggatttaaggttggagattcatgagaaccttggttttccttttctgtg
ctttctgcatgttttctgtacttcctttctcttcacccaaacaattagtggaattggcaaaagaagaagacaaagccacc  5-4400
ccaaccggTTTCTGGGACTTTGTTTCCTGCAGTTTGTATTGCTGGTTGCTGTGCATGGCTCAAGGGTTCCATGTTCACAC
```

GAGGCGCAGCGAACACAGTGTTCACAGCCAGGAGAATCGCAGTAGAAGTCTGGTTTGCACTTGCACTTGGTATTCTGGGT

CAGGGTGCAGTTTGTTTCCACTTCTAAACCATGCTCTTCATCGCAGAGTGTGCATCTTCTGCATTTATCAGCATAATGGT

TCTTGTCCATGTACTCCTTCCCTTCTGTGCATGGGGCACAGGTTGGTGTACCCCCATTCATTTTGCAGTCCTCAACTTTT

TTTTTACCAGGTTGGCATGGTTGACAGCAAAATGGGCCTCCTTGATATAATCCTTCTGAGCAGTTTTTATCAGTTTCATG

AACCCGCCTCCTCAGCTTTAAACTCTCGGAGATGCTATTAGTACCTTGAGTATGAACTCTTAACTGTGAGCCAGCAAGCA

CCAGAGGCAGGACAGCCCAGATCCACACCATgGTGGCTTTACCAACAGTACCGGAATGCCAAGCTTGCGGCCGCTTAAGA

GCTGTAATTGAACCTGGGAGTGGACACCTGTGGAGAGAAAGGCAAAGTGGATGTCAGTAAGACCAATAGGTGCCTATCAG

AAACGCAAGAGTCTTCTCTGTCTCGACAAGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACTTAC ⌐69 – 5120

CTGCCCAGTGCCTCACGACCAACTTctgcaggaattcctggacagctcccagatgatcagtaaccgtggttgttatttct
gtgccgggcagtggagcctgggtagggggagctctgcctcagtgctttcagctaaaaatggggtgggaaccccCaggagg
cccgggccgccctggaagttccctttttctctctgttcttgggaagtcgattgagcaacagcggggtcaggtgaggctcc
ttcactaccgatgcacaccgagtgctGggggaggttctcttctctctcaggcccaacCccagggccccctgcctaggtccc
ggactctCactcttgacgcatgcgtggcttggtggtcccagtcagcaaacttggggtcccgttgcctgggaaagggagag
ggtactgggcatcgacgcctctgcttccacgaaagccttgtgaagaaaggatgggggcgcttttgtgcaggagaatgagg
cgcactgaggtgaactggccctcggggGcgcgtgtcccagatgtgtgtgcagggcctcctgatggccgcagccctcgtcc
ctgtgacccgcttggagctggcaccctgagtggtggcctcacCTTGTACTCACTCCCAGGTCACTGTCCtcgacGCGGCC

GCTCGAcgatAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAg

ctagcTTAAGTAACCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCGGAACAG

ATGGAACAGGCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCG ⌐75 – 6000.

TGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGA

CTACCCGTCAGCGGGGGTCTTTCAcatgcaGCATGTATCAAAATTAATTTGGTTTTTTTTCTTAAGTATTTACATTAAAT

GGCCATAgtttcGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAG

CCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCT

TTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCG

CTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGG

TAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGT

AAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAG

GTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCC

TGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTC

AGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG ⌐86 – 6480

TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG

CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATC

TGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGG

TGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGT

CTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTT

TTAAATTAAAAATGAAGTTTGCGCAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAAT

CAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGA

TACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCA

ATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTG

CCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCAC

GCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAA

AAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGC ⌐98 – 7040

FIG 12C

ACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGagtactcaaccaagtcattctgag

aatagtgtatgcggcgaccgagttgctcttgcccggcgtcaacacgggataataccgcgccacatagcagaactttaaaa
gtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacc
cactcgtgcacccaactgatcttcagcatctttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatg
ccgcaaaaaagggaataagggcgacacggaaatgttgaatactcatactcttccttttcaatattattgaagcatttat
cagggttattgtctcatgacattaacctataaaaataggcgt

FIG 13A

(2) Ahhhh: Survival construct

2.) Ahhhh: epsilon-cFas' (CD8 or mLyt2)-Ires-Hygro-BGHpolyA also in C12s backwards

```
atcacgaggcccttttcgtcttcaagaacagcttttgctcttaggagttttctaatacatccaaactcaaatatataaagc    60    80
atttgacttgttctatgccctagttattadtagtaatcaattacggggtcattagttcatagcccatatatggagttccg
cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgaccccgcccattgacgtcaataatgacgtatg
ttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagta
catcaagtgtatcatatgccaagtacgcccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagta
catgaccttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggtttggc
agtacatcaatgggcgtggatagcggtttgactcacggggatttccaagtctccacccattgacgtcaatgggagtttg
ttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcatgt
acggtgggaggtctatataagcagagctcaataaaagagcccacaacccctcactcggggcgccagtcctccgattgact
gagtcgcccgggtacccgtgtatccaataaaccctcttgcagttgcatccgacttgtggtctcgctgttccttgggaggg
tctcctctgagtgattgactacccgtcagcggggggtctttcatttggggggctcgtccgggatcgggagacccctgcccag
ggaccaccgacccaccaccgggaggtaagctggccagcaacttatctgtgtctgtccgattgtctagtgtctatgactga
ttttatgcgcctgcgtcggtactagttagctaactagctctgtatctggcggacccgtggtggaactgacgagttcggaa    4    1120
caccggccgcaaccctgggagacgtcccagggacttcggggggccgtttttgtggcccgacctgagtccaaaaatcccga
tcgttttggactctttggtgcaccccccttagaggagggatatgtggttctggtaggagacgagaacctaaaacagttcc
cgcctccgtctgaattttttgctttcggtttgggaccgaagccgcgccgcgcgtcttgtctgctgcagcatcgttctgtgt
tgtctctgtctgactgtgtttctgtatttgtctgaaaatatgggcccgggccagactgttaccactcccttaagtttgac
cttaggtcactggaaagatgtcgagcggatcgctcacaaccagtcggtagatgtcaagaagagacgttgggttaccttct
gctctgcagaatggccaacctttaacgtcggatggccgcgagacggcacctttaaccgagacctcatcacccaggttaag
atcaaggtctttttcacctggcccgcatggacacccagaccaggtcccctacatcgtgacctgggaagccttggctttga
ccccctccctgggtcaagccctttgtacacccctaagcctccgcctcctcttcctccatccgccccgtctctccccttg
aacctcctcgttcgacccccgcctcgatcctcccttttatccagccctcactccttctctaggcgcccccatatggccatat
gagatcttatatggggcaccccgccccttgtaaacttccctgaccctgacatgacaagagttactaacagcccctctct
ccaagctcacttacaggctctctacttagtccagcacgaagtctggagacctctggcggcagcctaccaagaacaactgg    2000  -25
accgaccggtggtacctcacccttaccgagtcggcgacacagtgtgggtccgccgacaccagactaagaacctagaacct
cgctggaaaggacctacacagtcctgctgaccacccccaccgccctcaaagtagacggcatcgcagcttggatacacgc
cgcccacgtgaaggctgccgacccggggggtggaccatcctctagactgccGGATCTCGAGGGATCCTCCCCAGCATGCC

TGCTATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAA

TGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGG

GCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGtCTAGCTTGCCAAACCTACAGGTGGGGTCTTTCATTCCC

CCCTTTTTCTGGAGACTAAATAAAATCTTTTATTTTatcgatagatcccggtcggcatctactctattcctttgccctcg
gacgagtgctggggcgtcggtttccactatcggcgagtacttctacacagccatcggtccagacggccgcgcttctgcgg
gcgatttgtgtacgcccgacagtcccggctccggatcggacgattgcgtcgcatcgaccctgcgcccaagctgcatcatc  - 32
gaaattgccgtcaaccaagctctgatagagttggtcaagaccaatgcggagcatatacgcccggagccgcggcgatcctg
caagctccggatgcctccgctcgaagtagcgcgtctgctgctccatacaagccaaccacggcctccagaagaagatgttg
gcgacctcgtattgggaatccccgaacatcgcctcgctccagtcaatgaccgctgttatgcggccattgtccgtcaggac
attgttggagccgaaatccgcgtgcacgaggtgccgacttcggggccccaaagcatcagctcatcgagag
cctgcgcgacgacgcactgacggtgtcgtccatcacagtttgccagtgatacacatggggatcagcaatcgcgcatatg
aaatcacgccatgtagtgtattgaccgattccttgcggtccgaatgggccgaacccgctcgtctggctaagatcggccgc
agcgatcgcatccatggcctccgcgaccggctgcagaacagcgggcagttcggtttcaggcaggtcttgcaacgtgacac
cctgtgcacggcgggagatgcaataggtcaggctctcgctaaattccccaatgtcaagcacttccggaatcgggagcgcg
gccgatgcaaagtgccgataaacataacgatctttgtagaaaccatcggcgcagctatttacccgcaggacatatccacg
ccctcctacatcgaagctgaaagcacgagattcttcgccctcgacagctgcatcaggtcggagacgctgtcgaacttt
cgatcagaaacttctcgacagacgtcgcggtgagttcaggcttttttcatggtattatcatcgtgtttttcaaaggaaaac  — 44
cacgtcccgtggttcgggggcctagacgtttttttaacctcgactaaacacatgtaaagcatgtgcaccgaggcccag
atcagatcccatacaatggggtaccttctgggcatccttcagccccttgttgaatacgcttgaggagagccatttgactc
tttccacaactatccaactcacaacgtggcactggggttgtgccgcctttgcaggtgtatcttatacacgtggctttgg
ccgcagaggcacctgtcgccaggtggggggttccgctgcctgcaaagggtcgctacagacgttgtttgtcttcaagaagc
ttCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGAGGGGAAAGACCCctagactaga

ccaagctttggatttcatttctgaagtttgaattttctgagtcactagtaatgtccttgaggatgatagtctgaattttc
tctgcaagagtacaaagattggctttttttgagatctttaatcaatgtgtcatacgcttctttctttccatgaagttgatg
ccaattacgaagcagttgaactttctgttctgctgtgtcttggacattgtcattcttgatctcatctattttggcttcat
tgacaccattctttcgaacaaagcctttaacttgacttagtgtcatgactccagcaatagtggtgatatatttactcaag  - 53
```

tcaacatcagatataaatttattg␣␣␣␣ctgtttcaggatttaaggttggagattcatgag␣␣␣␣cttggtttttcctttctgtg
ctttctgcatgtttttctgtacttcctttctcttcacccaaacaattagtggaattggcaaaagaagaagacaaagccacc
ccaaccggtttccggtcccccttcactgagccacggggccgacaatcttctggtctctggggctgagatgtcccggtaggg
tgcacaggtgagggagttcgcagcactggcttggtagtagtagagttcactttctgaaggactggcacgacagaactgaa
gtacatcaccgagttgctgatgactgagcagaaatagtagccttcgttttccttgctgaacttgttcagggtgagaacgt
acttattattcgtgtccctcatggcagaaaacagtttcgacgaattcagcttctcgtcccacgttatcttgttgtgggat
gaagccatatagacaacgaaggtgggctgggggagtttggagctggagttctggaagagccaagagcatccttgcgaaac
ggaccccaacacttcacataccaggtccaccttctgaccaagttcggcgtccatttttctttggaaagattcggagttcgg
gtgcctgtggcttagcttctccactcccccaggataatcgactcacccagcagcagcaggttcagcgacagaaagcgggtc
aacggtggaggccatgGTGGCTTTACCAACAGTACCGGAATGCCAAGCTTGCGGCCGCTTAAGAGCTGTAATTGAACCTGG

GAGTGGACACCTGTGGAGAGAAAGGCAAAGTGGATGTCAGTAAGACCAATAGGTGCCTATCAGAAACGCAAGAGTCTTCT

CTGTCTCGACAAGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACTTACCTGCCCAGTGCCTCACG

ACCAACTTctgcaggaattcctggacagctcccagatgatcagtaaccgtggttgttatttctgtgccgggcagtggagc
ctgggtagggggagctctgcctcagtgctttcagctaaaaatggggtgggaaccccCaggaggcccgggccgccctggaa
gttccctttttctctctgttcttgggaagtcgattgagcaacagcgggggtcaggtgaggctccttcactaccgatgcaca
ccgagtgctGgggggaggttctcttctctctcaggcccaacCccagggccctgcctaggtcccggactctCactcttgac
gcatgcgtggcttggtggtcccagtcagcaaacttggggtcccgttgcctgggaaagggagagggtactgggcatcgacg-7b
cctctgcttccacgaaagccttgtgaagaaaggatgggggcgcttttgtgcaggagaatgaggcgcactgaggtgaactg
gccctcggggGcgcgtgtcccagatgtgtgtgcaggcctcctgatggccgcagccctcgtccctgtgaccgcttggag
ctggcaccctgagtggtggcctcacCTTGTACTCACTCCCAGGTCACTGTCCtcgacGCGGCCGCTCGAcgatAAAATAA

AAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAgctagcTTAAGTAACCCA

TTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCGGAACAGATGGAACAGGCAATAAA

AGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCT

CTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGG

TCTTTCAcatgcaGCATGTATCAAAATTAATTTGGTTTTTTTTCTTAAGTATTTACATTAAATGGCCATAgtttcGTAAT

CATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGT

AAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCT

GTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGC

TCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACA

GAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT

GGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAG

GACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC - 85

CTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGT

TCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT

CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGT

GCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCC

AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCA

AGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAC

GAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAG

TTTGCGCAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT

CAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCA - 94

TCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGG

AAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAA

GTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATG

FIG 13C

GCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTT

CGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTA

CTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGagtactcaaccaagtcattctgagaatagtgtatgcggcga

ccgagttgctcttgcccggcgtcaacacgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaa
acgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaact
gatcttcagcatctttTactTTtcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaaaaaagggaata
agggcgacacggaaatgttgaatactcatactcttcctttTtcaatattattgaagcatttatcagggttattgtctcat
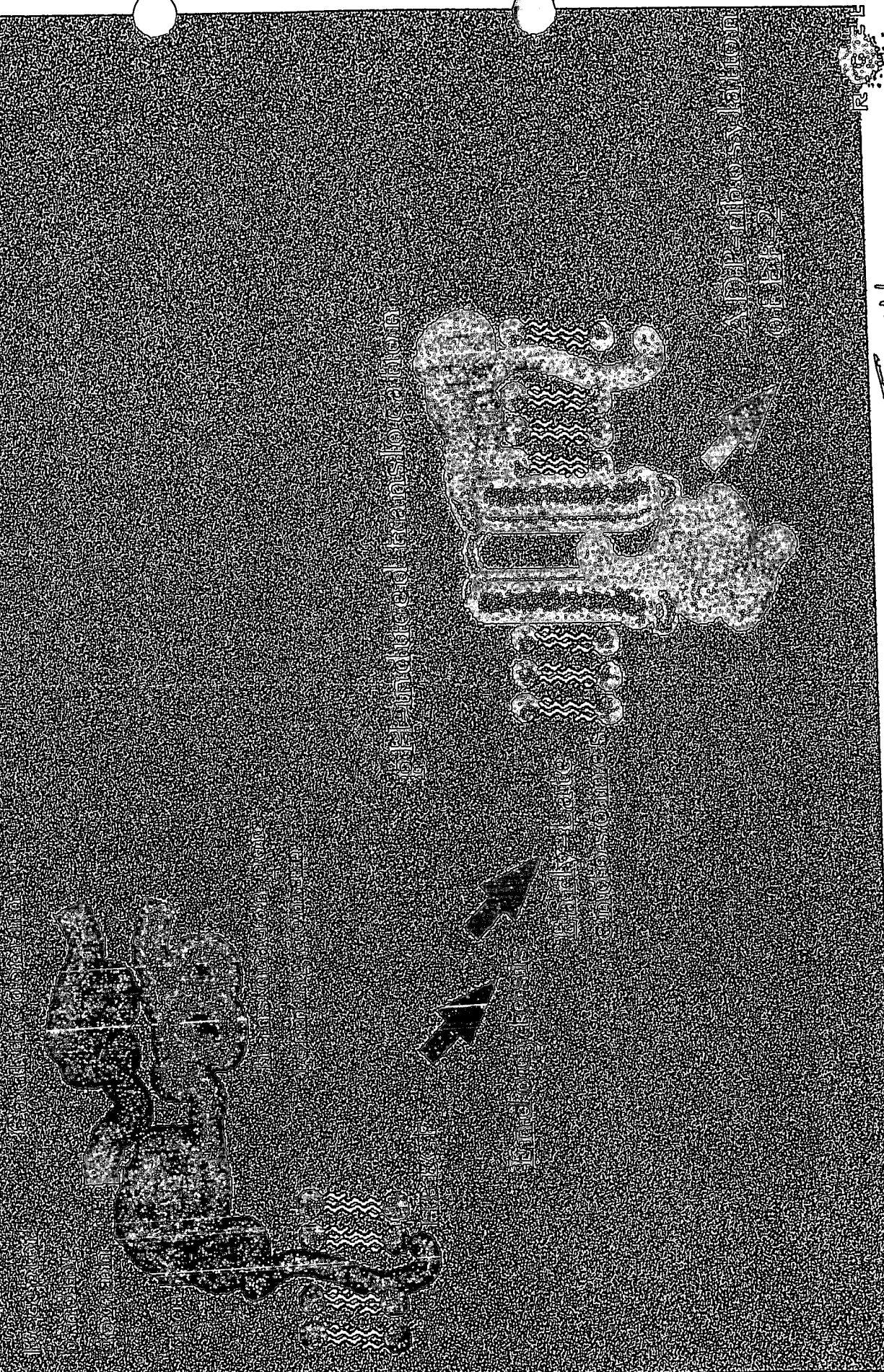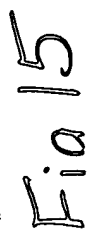gacattaacctataaaaataggcgt

Fig 14

Diphtheria toxin

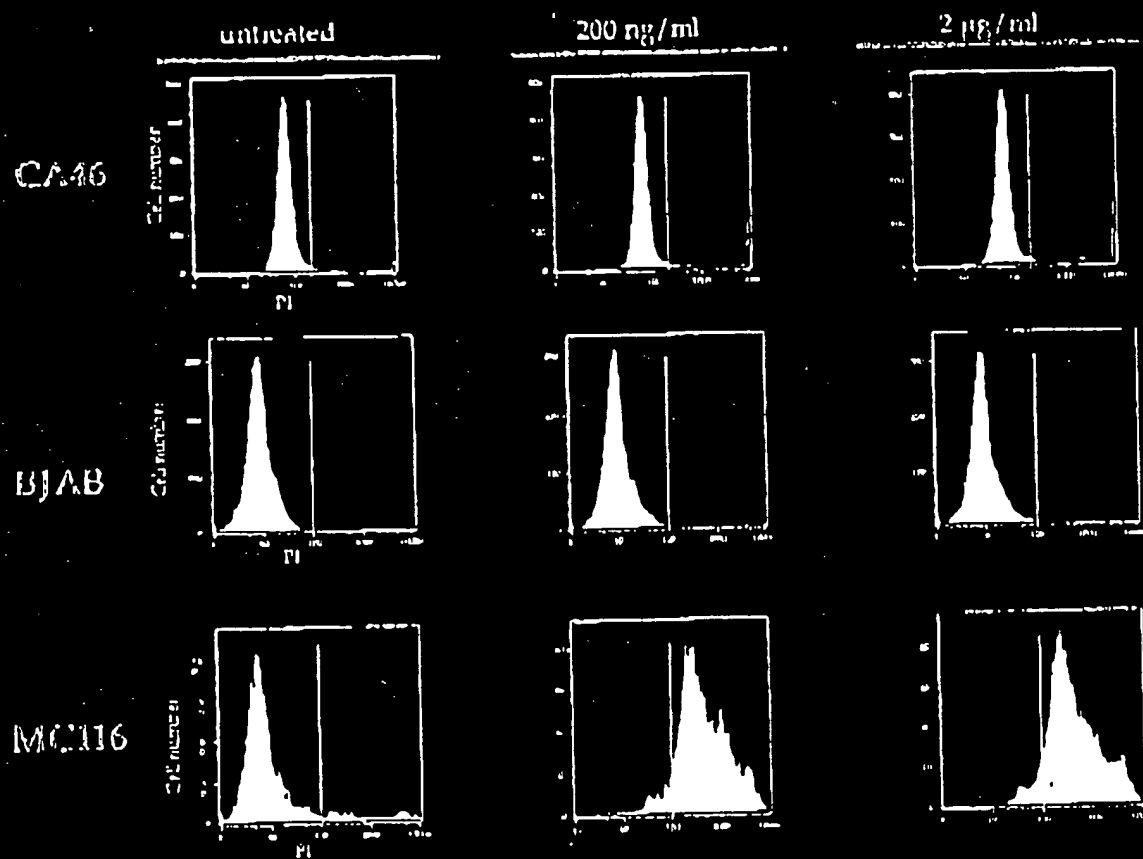ADP-ribosylation of EF2

ADP-induced translocation

Fig 15

HBEGF/Diphtheria as a selection tool

SELECTION

SOCS-1

PIM2

Jak3 Jak1

STAT6

IRS-1/2

PKCζ

SHIP

EXPRESSION

HBEGF

BGH
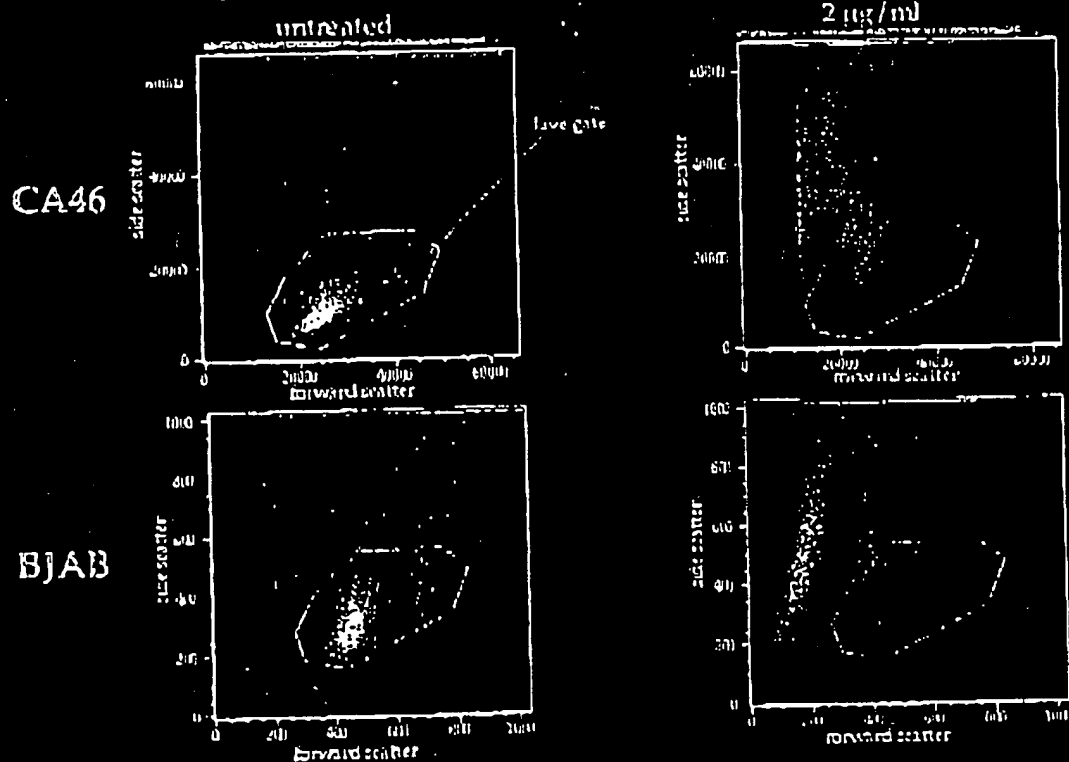polyA

2nd generation selection systems

Diphtheria sensitivity

FIG 16

2nd generation selection systems

Ectopic expression of HBEGF confers diphtheria sensitivity

Fig 17

Fig 18

A [LTR] — [BGH polyA] — [CD9] — [IRES] — [GFP 2A HBEGF] ⌄ [ε] — [LTR]

B [LTR] — [BGH polyA] — [CD9] — [IRES] — [HBEGF] ⌄ [ε] — [LTR]

[LTR] — [HBEGF | 2A | GFP] — [IRES] — [Puro^R] — [LTR] **C**

[LTR] — [HBEGF | GFP] — [IRES] — [Puro^R] — [LTR] **D**

[LTR] — [HBEGF] — [IRES] — [Puro^R] — [LTR] **E**

[LTR] — [GFP] — [IRES] — [HBEGF] — [LTR] **F**

[U3 | R | U5] — [HBEGF] — [IRES] — [Puro^R] — [U3 | R | U5] **G**